Hypothesis

Rewiring a receptor: negative output from positive input

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Abstract A point mutation or covalent modification in bacterial chemotaxis receptors causes bacteria to be repelled by attractants, and attracted to repellents. The variety of conditions causing inverse responses suggest that the signal transduction mechanism in receptors can be readily rewired to elicit inverse responses. A model is presented in which the orientation of a critical residue with respect to an active site determines whether the receptor produces normal or inverted signals. The model is consistent with observed responses and can be generalized to include receptors in other signal transduction systems.

Key words: Chemotaxis receptor; Aerotaxis; Signal transduction; *Escherichia coli*

1. Introduction

Receptors on the cell surface receive signals from the external environment and transmit information to the cytoplasm to effect appropriate responses. In type 1 receptors, a single transmembrane sequence links an extracellular sensing domain to an intracellular signaling domain [1]. Catalytic receptors have signaling domains that are either signal-transduction enzymes or regulatory subunits that form complexes with signaling enzymes. The catalytic domains may be protein histidine kinases in bacteria, or protein tyrosine kinases, serine-threonine kinases, guanylyl cyclases or protein phosphatases in eukaryotes. A diverse array of sensory and signaling modules are observed in type 1 receptors but they may share a common mechanism of transmembrane signaling.

The bacterial aspartate chemoreceptor Tar has two transmembrane segments but probably has a similar mechanism to type 1 and other receptors in transmitting a signal that regulates a histidine kinase protein (CheA) [1]. A chimera composed of the sensing domain of Tar fused to the signaling domain of the human insulin receptor produces a receptor with tyrosine kinase activity regulated by aspartate [2]. It is likely then, that advances in understanding the signal-transduction mechanism in bacterial receptors will continue to provide insight into signal transduction in other receptors.

Mutations and covalent modification of bacterial chemotaxis receptors can reprogram the receptors so that attractants elicit a repellent response and repellents elicit an attractant response. Despite long-standing documentation of such inverse responses, no satisfactory mechanism has been proposed to explain how a receptor can be rewired by a point mutation or methylation of glutamyl residues. In this hypothesis we propose a mechanism that is consistent with the known inverse responses in chemotaxis, and may be generalized to predict mechanisms for rewiring receptors in other signal transduction systems.

It is possible that simple mutations could also rewire eukaryotic receptors so that agonists become inverse agonists. Such changes could not only provide clues to the mechanism of signal transduction but also suggest an adaptational pathway by which cellular responses to an environmental signal can diversify.

2. Signal transduction in *Escherichia coli* chemotaxis

Escherichia coli, like other bacteria, migrate toward favorable microenvironments that contain nutrients and away from unfavorable microenvironments that are harmful to growth [3]. The receptors for chemotaxis, the products of the *tsr, tar, trg* and *tap* genes, are homodimers that have a common topology [3]. Each subunit has a sensor ectodomain that confers specificity on the receptors, two transmembrane helices and a cytosolic signaling domain that is highly conserved in all four receptors (Fig. 1). The signaling domain forms a complex with the CheA histidine kinase and CheW docking protein, and regulates autophosphorylation of CheA in response to chemoeffector binding to the receptor [4]. The phosphoryl residue is transferred from CheA to the CheY response regulator [5]. Phosphorylated CheY binds to a switch on the flagellar motors and triggers a change in the direction of motor rotation (Fig. 1).

Attractants bind to the receptor ectodomain inducing a conformational change in the signaling domain that inhibits CheA autophosphorylation [4]. Repellent binding induces a conformational change that activates phosphorylation of CheA. The excitation phase of the bacterial chemotaxis response produces a rapid change in tumbling frequency, is followed by a slower adaptation phase in which the bacteria adapt to the attractant or repellent stimulus by returning the tumbling frequency to the random tumbling observed in unstimulated cells. This is accomplished by adjusting the extent of receptor methylation [6,7]. *E. coli* cells adapt to an attractant stimulus by methylating glutamyl residues at K1 and R1 sites that flank the histidine kinase regulator domain (Fig. 1). Methylation presumably returns the regulator domain and histidine kinase activity to the pre-stimulus state. Cells adapt to repellents, or removal of attractant, by demethylation of glutamylmethyl esters in K1 and R1.

Transport or metabolism of the chemoeffector is not required in the above pathways for chemotaxis where chemicals bind to specific chemoreceptors [8]. Other behavioral re-

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sponses depend on transport or metabolism of the chemoeffector [9,10]. For example, in aerotaxis, the migration of bacteria to an optimal concentration of oxygen, the Aer and Tsr aerotaxis transducers detect changes in redox or the proton motive force, and not oxygen per se [11].

3. Examples of inverse chemotaxis responses

The inverse chemotaxis responses in *E. coli* and the closely related *Salmonella typhimurium* fall into three subclasses. (1) The inverse responses of *fliM* mutants do not involve receptor rewiring but are caused by a defect in the flagellar motor switch [12]. They will not be discussed further. (2) *E. coli* cells with specific mutations in Tsr, the serine receptor for chemotaxis, respond to weak acids as attractants rather than repellents [13,14]. (3) Deletion of the *cheB* (protein methylesterase) gene in *E. coli* results in overmethylation of Tsr, which similarly inverts the response to weak acids [14] and to oxygen [15]. Post-translational modification of the methylation sites changes the Tar receptor from a cold-preferring to a warm-preferring sensor [16]. Thus, a simple mutation or methylation rewires the receptor so that signaling is 180° out of phase. Models that propose a mechanism of signal transduction by the chemotaxis receptor should incorporate a mechanism for these inverse responses.

4. Transduction mechanism

The ectodomain of the Tar chemotaxis receptor consists of a four-helix bundle in each subunit [17] (Fig. 1). Aspartate binds across the interface between the subunits, and displaces the α_4 helix and second transmembrane domain [17,18]. It is likely that signal transduction is similar in Tsr and other chemoreceptors. Two models have been advanced for transmittal of the ligand-induced conformational change to the histidine kinase regulator region of the endodomain. In the piston model, the inward displacement of the second transmembrane domain translates the endodomain K1 helix, changing the conformation of the regulator domain [18,19]. In the rotational model, rotation of the K1 coiled-coil domain of the endodomain exposes residues in the signaling region that are critical for inhibition of CheA [20].

The available evidence does not conclusively validate either of the above models for signal transduction. In this hypothesis, we arbitrarily describe inverted responses in terms of the rotation model because we found it easier to visualize hypothetical schemes for the rotation model. Our model for inverted responses can be generalized to include the piston and other mechanisms for sensory transduction.

5. Schematic representation of signaling

Our studies of signal transduction in *E. coli* have investigated signaling by the Tsr and Aer proteins [11,15]. The signaling hypothesis is described for the Tsr receptor. Fig. 2A is a cartoon showing signaling by an unmodified Tsr receptor in a rotational model. In the ground state, a residue in Tsr that is critical for activating CheA is displaced from the CheA active site (Fig. 2A). Thermal energy randomly rotates the Tsr and CheA/W domains and occasionally the critical residue enters the active site, briefly activating CheA. This stochastic process in 1600 Tsr receptors per *E. coli* cell [21] results in a low steady state level of CheA activity in unstimulated cells, yielding a random-walk motility. As the mean orientation of the Tsr signaling domain is rotated clockwise (CW) to bring the critical residue closer to the active site of CheA, the probability of CheA activation is increased (Fig. 2B). Similarly, if the signaling domain is rotated counter-clockwise (CCW) the probability of CheA activation is decreased. Therefore, in this scheme a conformational change induced by repellent binding to the receptor rotates the signaling domain CW, producing continuous activation of CheA and a constantly tumbling bias in swimming (Fig. 2B, lower right).

Adaptation occurs when demethylation rotates the signaling domain CCW to restore the unstimulated orientation. Attractants induce a conformational change that rotates the signaling domain CCW, greatly reducing the probability of CheA activation and the cells swim smoothly without tumbling. The cells adapt to the attractant when CW rotation induced by methylation of Tsr returns the signaling domain

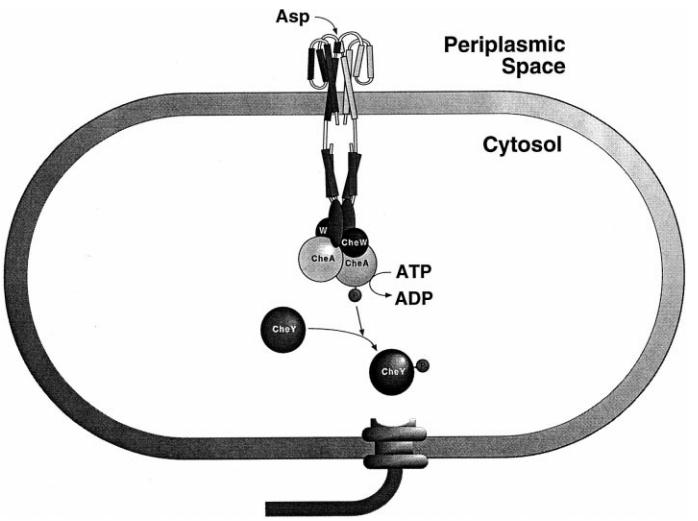

Fig. 1. Scheme of the signal transduction pathway for chemotaxis to aspartate in *E. coli*. Swimming behavior changes from smooth to tumbly when CheY-P binds the flagellar motor switch. CheY-P levels are controlled by CheY-P, a histidine autokinase that is modulated by the Tar receptor.

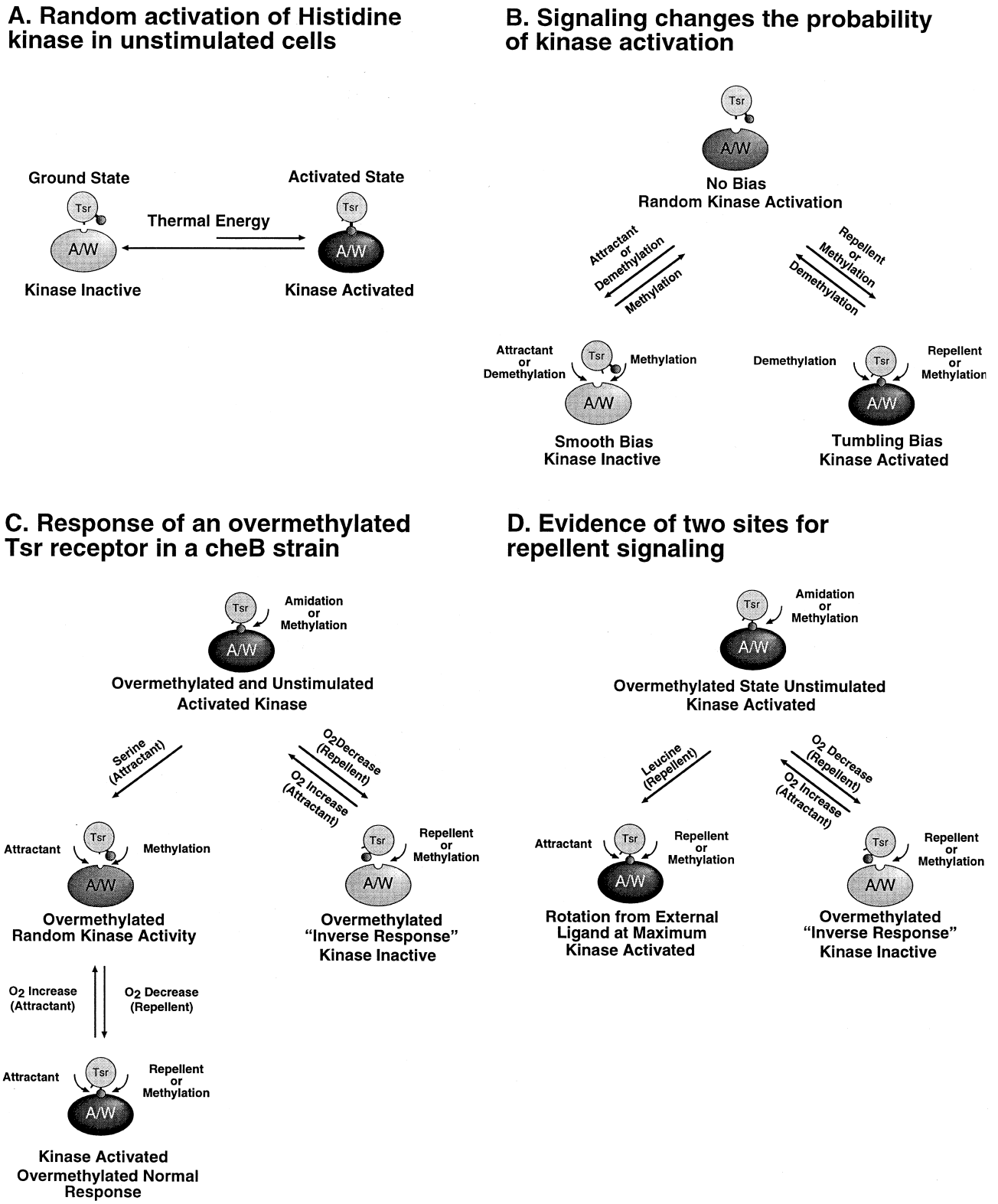

Fig. 2. Hypothetical conformational states of the Tsr/CheA/CheW complex in a rotational model for histidine kinase activation in *E. coli* chemotaxis. A cross section through the protein complex showing a critical residue is represented. Abbreviations: A, CheA histidine kinase; W, CheW docking protein.

to the unstimulated position. To summarize the scheme, repellents and methylation rotate the signaling domain CW and attractants and demethylation rotate the domain CCW. Nascent chemotaxis receptors are amidated at some of the γ-glutamyl methylation sites and must be deamidated before the sites can be methylated [22]. In this scheme, amidation and methylation of these sites cause a similar CW rotation of the signaling domain.

6. Rewiring a receptor: the hypothesis

The CheB protein is a protein methylesterase that deamidates and demethylates the chemotaxis receptors [22,23]. In a CheB mutant, the receptors are methylated and amidated, even in the unstimulated state. In Fig. 2C, the covalent modification of the receptor rotates the signaling domain CW to the 6 o'clock position where CheA is constantly activated and the bacteria tumble constantly. Under conditions where the receptor is extensively modified, it is proposed that repellents, such as a decrease in oxygen concentration or an acid shift in cytoplasmic pH, can force a CW twist beyond the 6 o'clock position toward the 7 o'clock position (Fig. 2C, top right). This moves the critical residue out of the active site and CheA is no longer fully activated. The result is a paradoxical smooth (attractant) response to the addition of a repellent. When oxygen (attractant) is added, it is proposed that the signaling domain returns to the 6 o'clock position and a tumbling (repellent) response is observed.

In this hypothesis, wiring of the signal transduction mechanism of the receptor for normal or inverse responses depends on the orientation, in the unstimulated state, of the critical residue relative to the active site. Similar conformational changes induced in the receptor by attractants (or repellents) produce opposite results depending on whether the critical residue is moved toward, or away from, the active site.

7. Testing the hypothesis

The hypothesis is consistent with our experimentally observed inverse responses to oxygen in CheB mutants of *E. coli* [15]. An increase in oxygen concentration causes a repellent response in CheB mutants, but an attractant response in wild-type cells. The inverse responses are transduced by Tsr which senses the proton motive force or rate of electron transport [11,15]. The model predicts that other conditions that alter electron transport/proton motive force will also elicit inverse responses in *cheB* cells. This has been confirmed for substituted quinones, redox molecules that short circuit the electron transport system [24], and glycerol taxis in which the supply of reducing equivalents to the electron transport system is affected [25].

Pretreatment of the *cheB* cells with 10 μM serine restores to normal the response to an oxygen decrease or increase (unpublished observations). The hypothesis is consistent with this switch in signal orientation. The signaling domain is rotated CW by overmethylation, but serine induces a compensating CCW rotation of the domain and reorients the signaling domain to approximately the 5 o'clock position (Fig. 2C, middle left). This restores random swimming. An oxygen decrease (repellent) would move the critical residue CW toward 6 o'clock, increasing activation of CheA and tumbling (repellent response) in the serine-treated *cheB* cells (2C, lower left). An oxygen increase (attractant) would shift the critical residue toward 4 o'clock, decreasing CheA activation and causing smooth swimming (attractant response). Attractant adapted *cheB* cells give responses to oxygen (Fig. 2C) that are predicted by the model (unpublished observation).

According to this model, other repellents should also elicit an inverse response in *cheB* cells (Fig. 2D). However, leucine which apparently binds to the ectodomain of Tsr, does not elicit a response in *cheB* cells (M. Johnson, unpublished observations) or in Tsr cells that show an inverted response to weak acids [13]. The model can be modified to accommodate

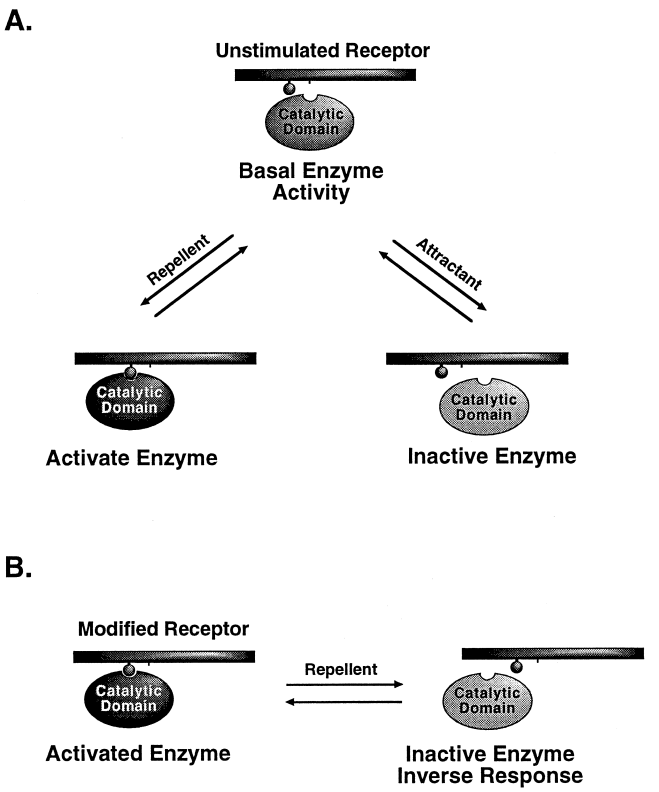

Fig. 3. Generalized linear displacement model for receptor signaling showing normal (A) and inverse (B) signaling. See text for description.

this finding by postulating two pivot points in Tsr. CW rotation of the coiled-coil domains around the first pivot point in response to leucine binding to the ectodomain is more constrained. Rotation around a second pivot point, that is *internal* to the first pivot point, in response to methylation and periplasmic signals is less constrained.

8. Generalized hypothesis

The inverse response hypothesis has been described in terms of the rotational model for signal transduction in bacterial chemotaxis. The hypothesis can be adapted readily to other signal transduction models. For example, in the linear displacement (piston) model for aspartate chemotaxis, inward displacement by aspartate of the α_4 helix and second transmembrane domain moves the K1 coiled-coil domain inward, repositioning a critical residue with respect to the active site of CheA. The critical residue is shown to the left of the active site in unstimulated cells that have normal signaling responses (Fig. 3A). Displacement of the signaling peptide to the right will increase catalytic activity and displacement to the left will decrease the activity. A modified receptor places the critical residue at the active site in unstimulated cells. Displacement to the right by repellent binding will effect an inverse response (Fig. 3B).

This extension of the inverse response hypothesis can be generalized for other models of signal transduction. Any conformational change that moves a critical residue toward an active site will move the residue away from the active site if the residue starts at, or on the distal side of, the active site. This principle is not confined to chemoreceptors or to bacterial receptors. It is applicable to sensory receptors in vertebrates or plants. As the three-dimensional structure becomes available for increasing numbers of sensory receptors, a careful comparison of the signaling domains will be able to determine the role of such a mechanism in the evolution of a diversity of receptor responses. It may also be applicable to other forms of cellular regulation as it provides a simple mechanism by which allosteric activators of enzyme activity could become allosteric inhibitors of the enzyme activity after mutation of the enzyme. Extensive testing will be required for validation, but this hypothesis provides a model for signal transduction that accounts for observed inverse responses. A thermodynamic model for inversion of photoresponses in Halobacterium salinarum was recently proposed [26].

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